

IN THE CLAIMS:

Please amend claims 1-9, 11, 13, 14 and 26 and add new claims 27 and 28 as follows:

1. (Currently Amended) A method for examining nucleotide sequences which comprises:
 - (1) adding a group of primers consisting of multiple primer species to a solution containing a sample of nucleic acid subjected to examination, and performing simultaneous synthesis of complementary strands at each of the multiple regions of the nucleic acid containing target nucleotide sequences to be examined;
 - (2) designing DNA probes with specific sequences so that elongation of complementary strands is affected by the presence or absence of mutations in said target nucleotide sequences wherein the same number of such DNA probes and said target sequences is used for elongation of complementary strands;
 - (3) immobilizing said DNA probes in subcells of a reaction vessel that are compartmentalized for each said DNA probe;
 - (4) adding the solution obtained after step (1) to the reaction vessel, wherein said DNA probes are allowed to hybridize with said target sequences or sequences complementary to said target sequences under condition that the solution can freely move among said subcells, and then the excess amount of the solution is removed so that the remaining solution can no longer freely move among said subcells;
 - [[(3)]] (5) performing elongation reaction of complementary strands using said ~~targets~~ target sequences or the sequences complementary to said ~~targets~~ target sequences as a template and the following reaction, in which pyrophosphate produced during said elongation reaction is converted to ATP and reacted with chemiluminescent substrates to develop luminescence, in the subcells of the reaction vessel ~~that are compartmentalized for each said target, wherein elongation of complementary strand occurs for the nucleic acid whose sequences are complementary to said probes;~~ and
 - ~~(4) detecting said luminescence,~~ (6) determining the presence or absence of mutations present in said target nucleotide sequences based upon said luminescence.

2. (Currently Amended) A method for examining nucleotide sequences according to claim 1, ~~which wherein the step (1) comprises:~~

~~(1) a group of said primers consisting of the~~ (a) adding first anchor primers each having an arbitrary a first anchor sequence at the 5' terminus that is not hybridized with said targets;

[[(2)]] (b) removing excess of said first anchor primers after said synthesis of complementary strands, followed by using a group of the second anchor primers for synthesis of complementary strands; and

(3) these second anchor primers containing an arbitrary a second anchor sequence at the 5' terminus that is not hybridized with said targets and [[the]] a sequence complementary to a part of the complementary strands in (1) (b) synthesized by said complementary strand synthesis;

[[(4)]] (c) preparing DNA strands with said first or second anchor sequence at least at one terminus by using these second anchor primers; and

[[(5)]] (d) amplifying the number of copies of said DNA strands with said first or second anchor sequence as a priming region.

3. (Currently Amended) A method for examining nucleotide sequences according to claim 1 ~~which comprises:~~

[[(1)]] wherein in step (2), said DNA probes are hybridized with said targets designed so that the base at 3' terminus of said primers DNA probes is located exactly on said mutation sites by using single strands prepared from said amplified DNA strands as a template at a mutation site in said target nucleotide sequences ; and

[[(2)]] the elongation of complementary strands of said DNA probes depends on the base species at the putative mutation site.

4. (Currently Amended) A method for examining nucleotide sequences according to claim 1 ~~which comprises: wherein~~ [[a]] the group of said primers consists of the anchor primers having an arbitrary anchor sequence that is not hybridized with said targets at the 5' terminus; defining a 3' terminal region, and each of the anchor primers' [[are]] is designed so that ~~said mutation is located~~ it has a base at the expected site ~~when the 3' terminal region is hybridized to~~ for recognizing a mutation site in said targets; and the

elongation of complementary strands for each of said anchor primers is regulated by the presence of said mutations is dependent on the presence of mutation in said targets.

5. (Currently Amended) A method for examining nucleotide sequences according to claim 4 in which sequences of said anchor primers are varied so ~~that it is easy to distinguish the correspondence between each of said primers and the sequence of said targets and that~~ so as to provide complementarity between the 3'-terminal region and the DNA of a mutant or a wild type.
6. (Currently Amended) A method for examining nucleotide sequences according to claim ~~[[1]]~~ 2 in which ~~a group of said anchor primers consists of the anchor primers having the first arbitrary anchor sequence at the 5' terminus that is not hybridized with said targets, excess of said primers is removed after said synthesis of complementary strands, and the second anchor primer, in which the 5' terminal region contains an arbitrary second anchor sequence that is not hybridized with said targets, the remaining region contains the sequence complementary to a part of complementary strands produced by said synthesis of complementary strands, and the 3' terminal region is designed to be capable of hybridizing with said target sites of possible mutation, is used for the synthesis of complementary strands, the DNA strands thus obtained are used for synthesis of complementary strands by using the primers that are hybridized with either the complementary sequences of said first anchor or said second anchor sequences, and the copy number of DNA strands is amplified by either PCR amplification, rolling-cycle amplification or amplification using a loop structure.~~
7. (Currently Amended) A method for examining nucleotide sequences according to claim 1, in which said DNA probes contain the sequences specific to said target sequences and are immobilized onto the surface of said subcells, and said amplified DNA strands are ~~degenerated~~ denatured to prepare single strands, which are captured in said subcells by hybridization between this single-strand DNA and said DNA probes.
8. (Currently Amended) A method for examining nucleotide sequences according to claim ~~[[1]]~~ 2 in which said DNA probes contain the sequences specific to said target ~~species~~

nucleotide sequences and are maintained with matrix in said subcells, and said amplified DNA strands are ~~degenerated~~ denatured to prepare single strands, which are captured in said subcells by hybridization between this single-strand DNA and said DNA probes.

9. (Currently Amended) A method for examining nucleotide sequences according to claim ~~[[1]]~~ 2 in which said DNA probes contain the sequences specific to said target ~~species~~ nucleotide sequences and are maintained in said subcells, said amplified DNA strands are ~~degenerated~~ denatured to prepare single strands, which are captured in said subcells by hybridization between this single-strand DNA and said DNA probes, and synthesis of complementary strands is performed with ~~[[the]]~~ circular DNA added to said subcell as a template and by using the anchor sequence containing the 3' terminus of said single-strand DNA thus captured or of their complementary strands as a primer sequence.
10. (Original) A method for examining nucleotide sequences according to claim 9 in which the primers used for synthesis of complementary strands in each of said subcells are common to said multiple subcells.
11. (Currently Amended) A method for examining nucleotide sequences according to claim ~~[[1]]~~ 2 in which the anchor sequence containing the 3'-terminal region of said DNA strands captured in said subcells or their complementary strands is used as a primer to perform synthesis of complementary strands using ~~[[the]]~~ DNA with a loop structure separately added as a template.
12. (Original) A method for examining nucleotide sequences according to claim 9 in which the capture of DNA strands subjected to complementary strand synthesis is achieved by binding of said DNA strands to said DNA probes immobilized to the solid surface of said subcells or to the surface of beads placed in said subcells.
13. (Currently Amended) A method for examining nucleotide sequences according to claim ~~[[1]]~~ 2 in which pyrophosphate is produced during synthesis of complementary strands using the DNA strands ~~containing the sequence that is not related to said target DNA.~~

14. (Currently Amended) A method for examining nucleotide sequences according to claim 1 in which the probes used for preparing the first complementary strands from [[a]] the sample are the first anchor primers, whose anchor region is self-hybridized to form a loop-like complementary strand, which acts as templates for the second synthesis of complementary strands in said subcells by using [[the]] second anchor primers and the third primers, and pyrophosphate produced by formation of loop-like DNA strands in said second synthesis of complementary strands is converted to ATP, which is consequently utilized for chemiluminescent reaction.
15. (Withdrawn) A method for examining nucleotide sequences characterized by detection of mutations in genes or DNA with the following steps:
- (1) a group of probes consisting of multiple probe species is added to a sample solution subjected to examination;
 - (2) two said probes are hybridized with each of the different target sequences;
 - (3) the DNA probes are prepared so that the binding reaction of two said probes in ligation is affected by the presence of base mutations in DNA subjected to examination;
 - (4) using a group of said probes consisting of pairs of these probes, long DNA strands are prepared by ligation reaction of two said probes;
 - (5) synthesis of complementary strands is performed at least once in subcells of a reaction vessel by using either said DNA strand or their complementary strand as a template; and
 - (6) pyrophosphate, the product of complementary DNA synthesis in each of said subcells, is converted to ATP and reacted with chemiluminescent substrates to develop luminescence in said subcells compartmentalized for each of said target sequences.
16. (Withdrawn) A method for examining nucleotide sequences according to claim 15 in which:
- (1) each of said paired primers used in ligation used in ligation possesses the anchor sequence that is not hybridized to said target sequences at the 3' or 5' terminus;
 - (2) using said anchor sequences or their complementary strands, ligation products are PCR-amplified with said anchor sequences as a priming region; and

(3) all the subject DNA sites are simultaneously amplified and used as a template for synthetic reaction of complementary strands for chemiluminescent detection.

17. (Withdrawn) A method for examining nucleotide sequences according to claim 15 in which said amplified DNA strands are degenerated to prepare single strands, which are captured by the DNA probes that can recognize different specific sequences and are immobilized to subcells with the aid of the DNA sequences specific to said target sequence species, and synthesis of complementary strands for each of said target sequences is performed in said subcells.
18. (Withdrawn) A method for examining nucleotide sequences according to claim 15 in which:
 - (1) said amplified DNA strands are degenerated to prepare single strands;
 - (2) the DNA sequences specific to said target sequence species are divided into said subcells;
 - (3) in said subcells the probes are previously maintained with matrix;
 - (4) said probe in each said subcell contains the specific sequence that can recognize said different target sequence; and
 - (5) synthesis of complementary strands for each said target sequence is performed in each said subcell.
19. (Withdrawn) A method for examining nucleotide sequences characterized by the following point:

In the method for examining nucleotide sequences according to claim 15, using the anchor sequence containing the 3' terminus of said single-strand DNA thus captured in each said subcell or of their complementary strands as a primer sequence, synthesis of complementary strands is performed with the circular DNA added to said subcell as a template.
20. (Withdrawn) A method for examining nucleotide sequences according to claim 19 in which the primers used for synthesis of complementary strands in each of said subcells are common to at least said different subcells.

21. (Withdrawn) A method for examining nucleotide sequences according to claim 15 in which the anchor sequences containing 3'-terminal region of said DNA strands captured in said subcells or their complementary strands are used as a primer to perform synthesis of complementary strands using the DNA with a loop structure separately added as a template.
22. (Withdrawn) A method for examining nucleotide sequences according to claim 19 in which the capture of said DNA strands subjected to synthesis of complementary strands is achieved by the DNA probes immobilized to the solid surface of said subcells or to the surface of beads placed in said subcells.
23. (Withdrawn) A method for examining nucleotide sequences according to claim 15 in which pyrophosphate is produced during synthesis of complementary DNA strands containing the sequence that is not related to said target sequences.
24. (Withdrawn) A method for examining nucleotide sequences according to claim 15 in which:
- (1) a pair of probes used for preparing the ligation product from a sample are the anchor primers, whose anchor region is self-hybridized to form a loop-like complementary strand;
 - (2) the product DNA of said ligation by a pair of said probes acts as a template for synthesis of complementary strands using the third primers in subcells; and
 - (3) pyrophosphate is produced during formation of loop-like DNA strands in synthesis of complementary strands.
25. (Canceled)
26. (Currently Amended) A method for examining nucleotide sequences characterized by detection of specific sequences and mutations in base sequences of [[said]] target DNA, and ~~by the following steps the method~~, using a solution having genomes or multiple target DNA [[as a]] to provide templates, ~~in which comprising~~:

(1) hybridizing multiple species of [[the]] first probes ~~are hybridized~~ to said templates in a single reaction vessel ~~to prepare and preparing~~ multiple species of [[the]] first complementary strands by the first synthesis of complementary strands, wherein the first probes are immobilized in subcells of a reaction vessel that are compartmentalized for each of said probes, wherein said probes are allowed to hybridize with said target DNA under condition that the solution can freely move among said subcells; and then the excess amount of the solution is removed so that the solution can no longer freely move among said subcells;

(2) isolating and removing excess of the first probes ~~are isolated and removed~~ from said first complementary strands;

(3) with said first complementary strands as a template, performing the second synthesis of complementary strands ~~is performed~~ using multiple species of [[the]] second probes to obtain [[the]] second complementary strands, which partially contains the same sequence as that of said target DNA[[:]]

[[(4)]], whereby, in each compartmentalized area sorted with species of said first complementary strands, pyrophosphate is produced in the synthesis of said second complementary strands or in the complementary strand synthesis using said second complementary strands as a template, and is converted to ATP, which develops chemiluminescence for detection.

27. (New) A method of examining nucleotide sequences which comprises:

(1) providing a reaction vessel wherein a first probe consisting essentially of a first nucleotide sequence is immobilized in a first subcell of the reaction vessel, a second probe consisting essentially of a second nucleotide sequence different from the first nucleotide sequence is immobilized in a second subcell of the reaction vessel, and the first and second subcells are compartmentalized for each other;

(2) adding to the reaction vessel a solution containing a target nucleotide sequence in such a way that said solution can freely move between said first and second subcells, whereby the target nucleotide sequence hybridizes with said first or second probe;

(3) removing the excess amount of the solution so that remaining solution can no longer move between said first and second subcells;

(4) adding to the first and second subcells, reaction solutions necessary for complementary strand synthesis so that elongation reaction of the first probe or second probe is performed using said hybridized target nucleotide sequence as a template, and pyrophosphate produced during said elongation reaction is converted to ATP and reacted with chemiluminescent substrate to develop luminescence; and

(5) detecting said luminescence to detect a specific sequence in the target nucleotide sequence.

28. (new) A method for examining nucleotide sequence according to claim 27, wherein said first and second probes are for detecting wild-type or mutated target nucleotide sequence.